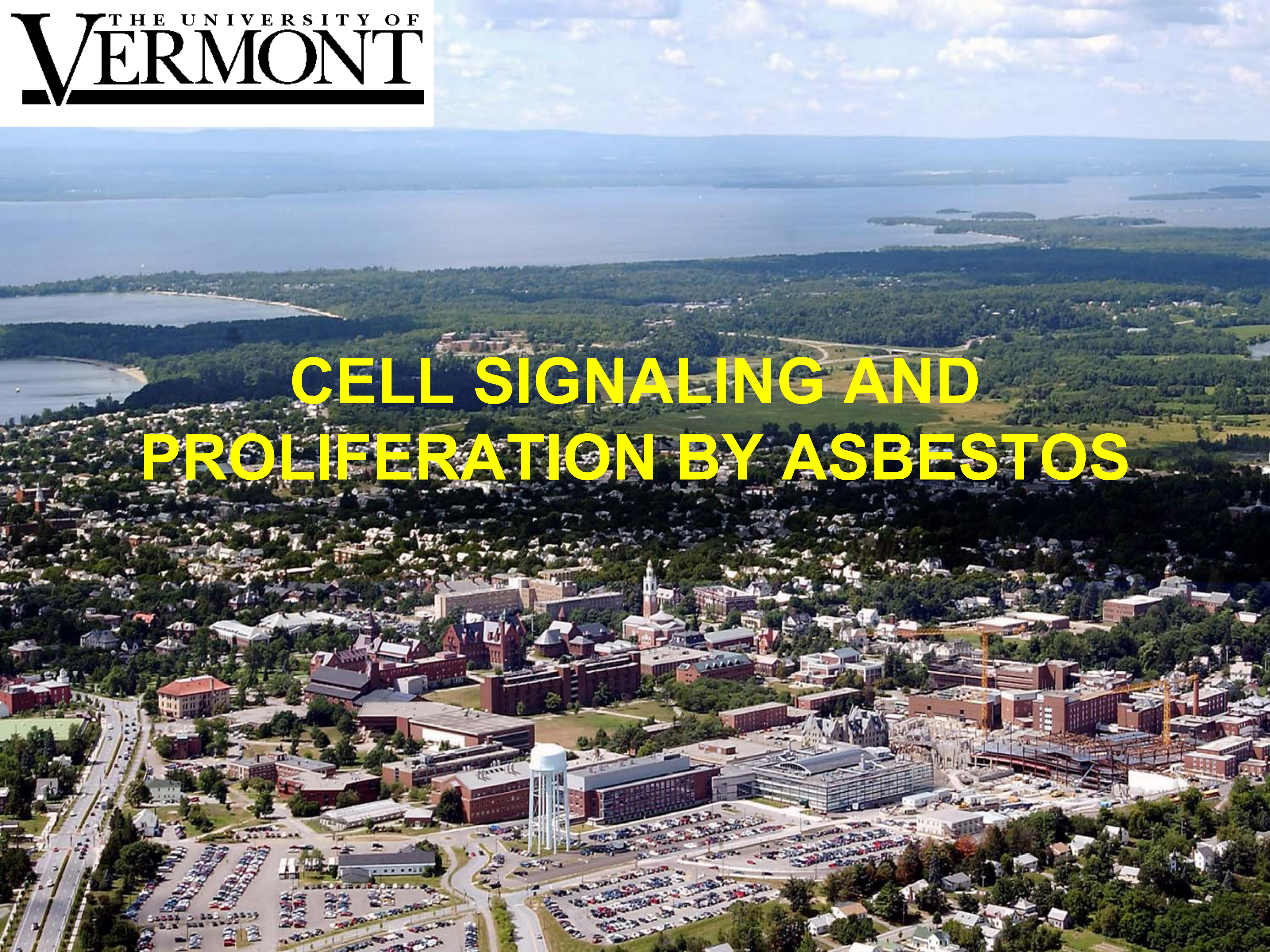
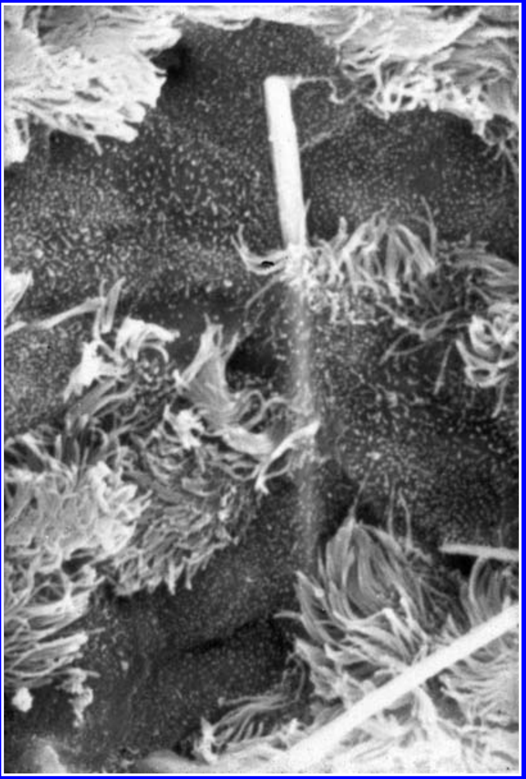
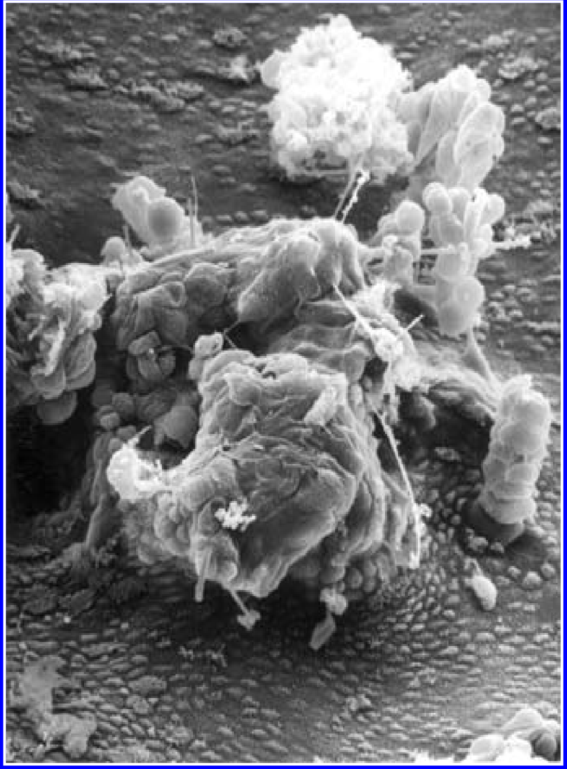
THE UNIVERSITY OF VERMONT



CELL SIGNALING AND PROLIFERATION BY ASBESTOS

DIFFERENT MORPHOLOGY OF CHRYSOTILE AND CROCIDOLITE FIBERS

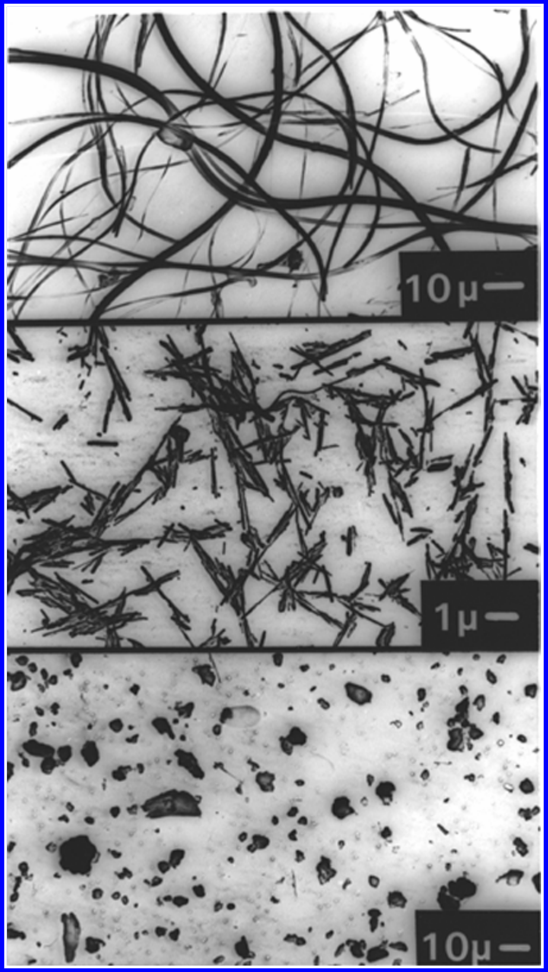




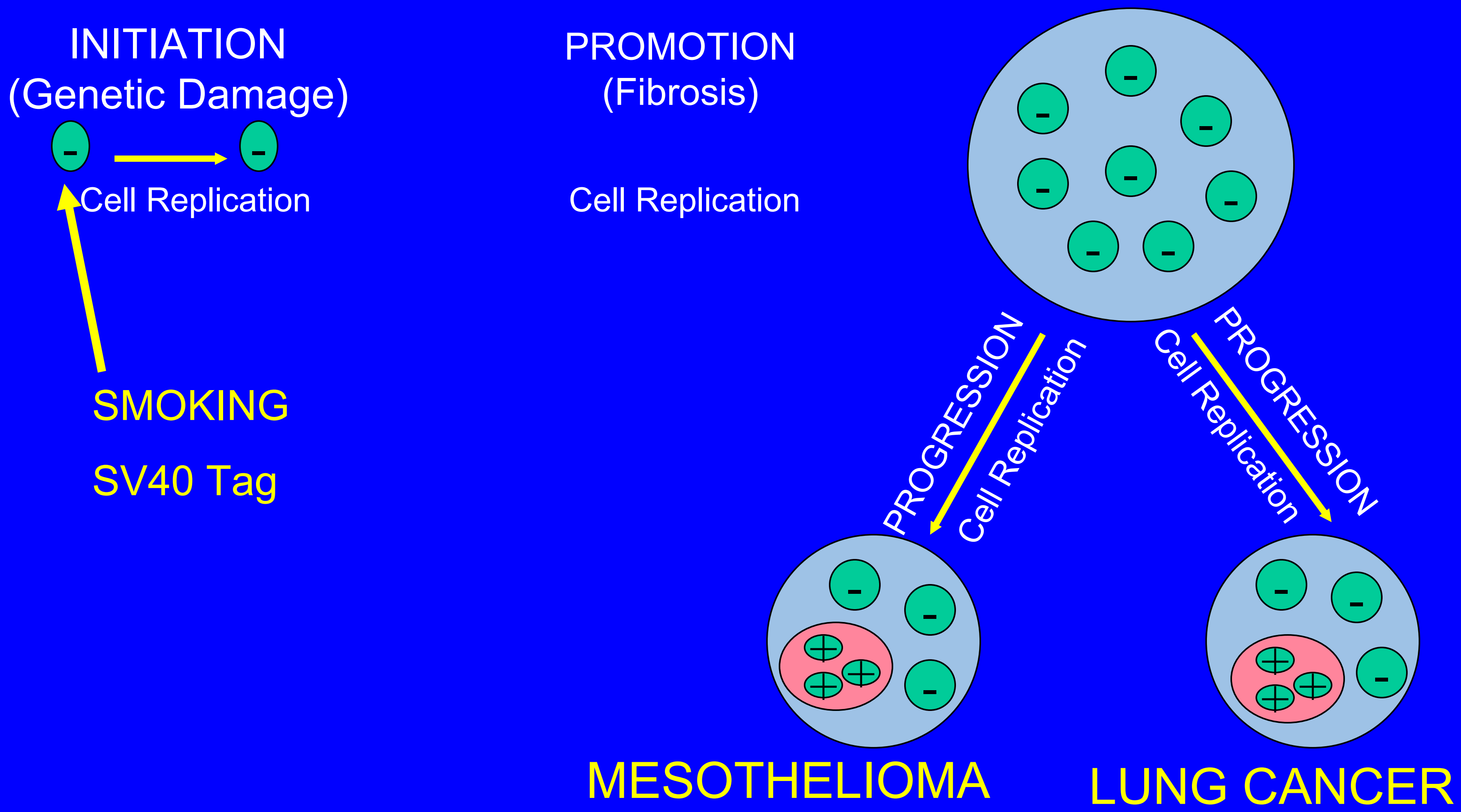
DIFFERENT TOXICITIES OF ASBESTOS FIBERS

- The cell membrane is the first target of asbestos fibers.
- Chrysotile asbestos (positively charged) is lytic to cells at high concentrations.
- Less toxic (neutral or negatively charged) asbestos (Crocidolite, Amosite) generates oxidants by iron-dependent reactions and frustrated phagocytosis.
- Long (>8microns) asbestos fibers are more toxic, mitogenic and pathogenic than short fibers.

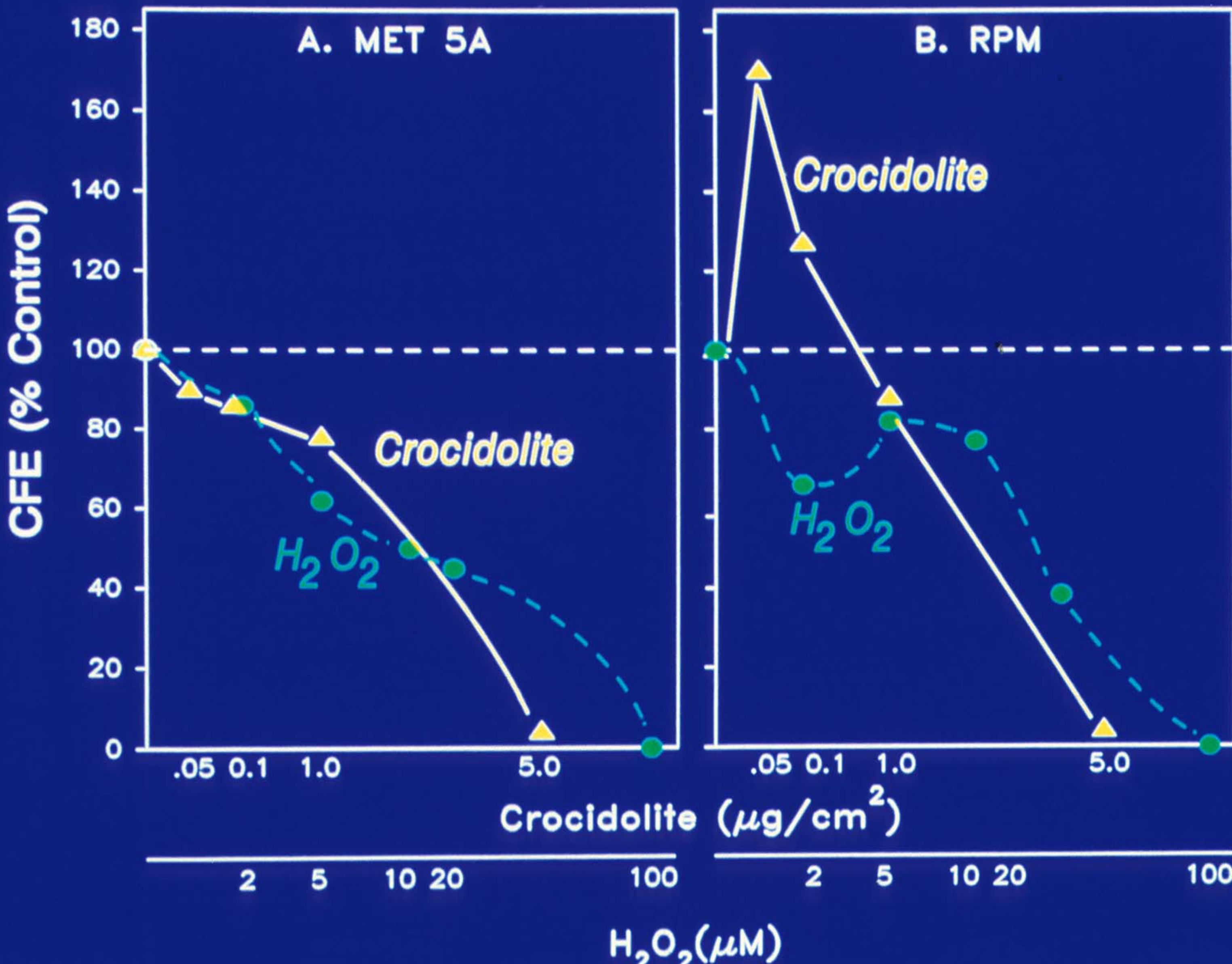
FIBERS VS. PARTICLES



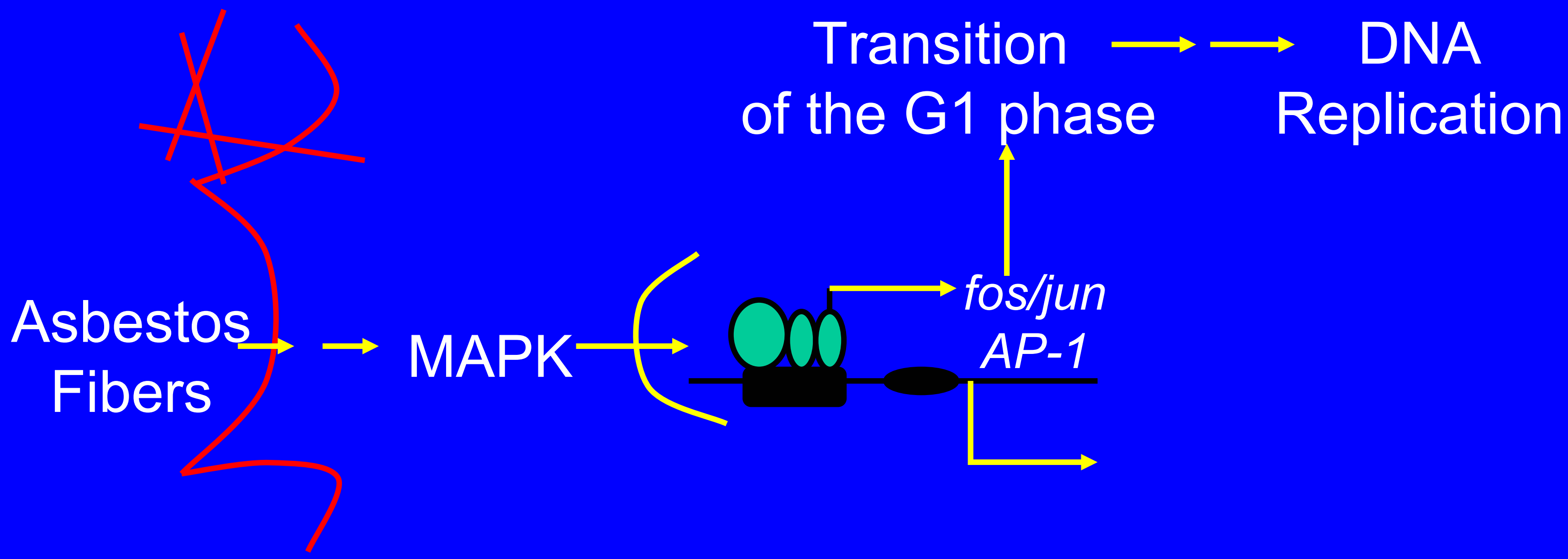
CELL PROLIFERATION AND PROTOONCOGENE EXPRESSION ARE HALLMARKS OF ASBESTOS-INDUCED CANCERS



COLONY FORMING ABILITY OF HUMAN MET 5A CELLS AND RAT PLEURAL MESOTHELIAL (RPM) CELLS FOLLOWING EXPOSURE TO ASBESTOS



MECHANISMS OF ASBESTOS-INDUCED PROLIFERATION



Signal Transduction → Persistant Induction of early response genes → Cell proliferation → Disease

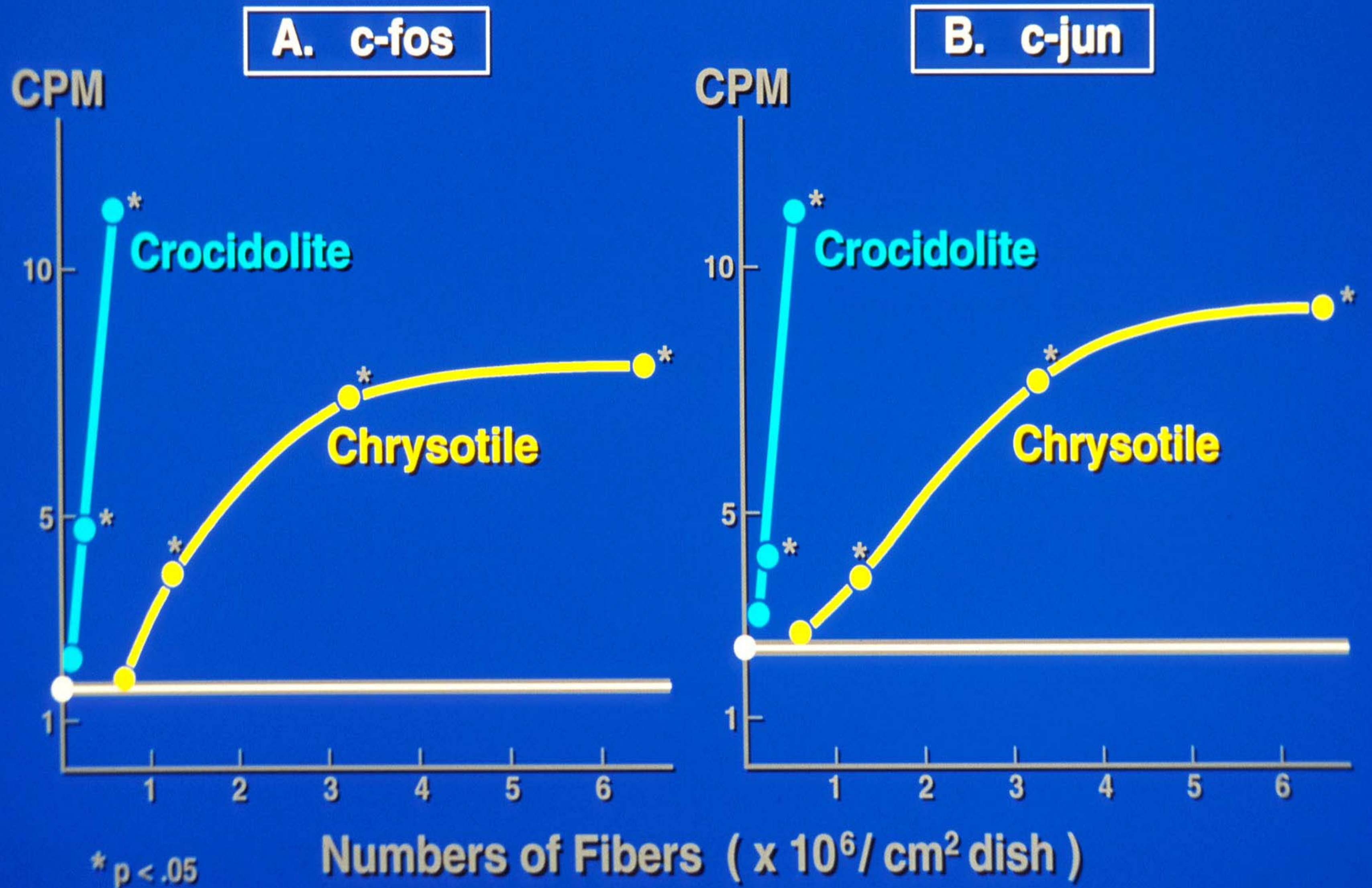
STRATEGIES FOR DETERMINING DOSE RESPONSE RELATIONSHIPS BY ASBESTOS TYPES

1. Gene expression (mRNA) studies in mesothelial/epithelial cells *in vitro* – *fos/jun* family.

2. Confirmatory studies using rat inhalation models
   - *c-jun** mRNA levels, *odc* mRNA levels, DNA synthesis (5'-BrdU)
   - Fibrosis (hydroxyproline)

*Timblin et al., Cancer Res. 55: 2723-6, 1995.

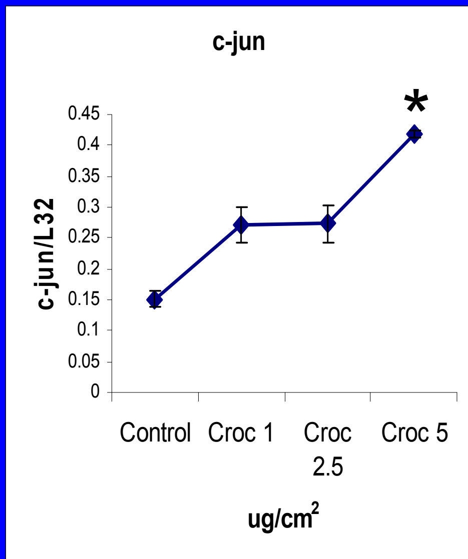
GENE EXPRESSION OF PROTOONCOGENES AFTER EXPOSURE OF RAT PLEURAL MESOTHELIAL (RPM) CELLS TO ASBESTOS

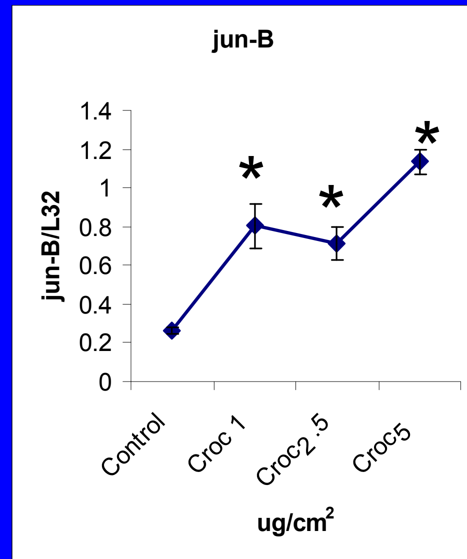


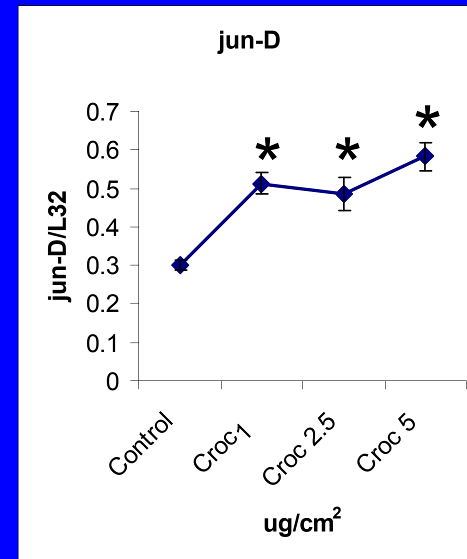
Heintz et al., PNAS USA 90: 3299-303, 1993.

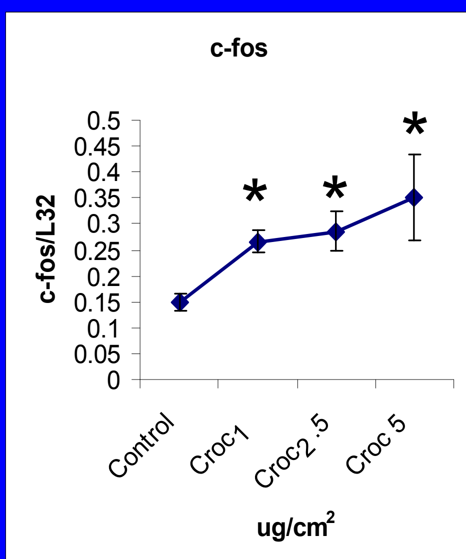
GENE EXPRESSION OF *fos/jun* PROTOONCOGENES AFTER EXPOSURE OF C10 LUNG EPITHELIAL CELLS TO CROCIDOLITE ASBESTOS

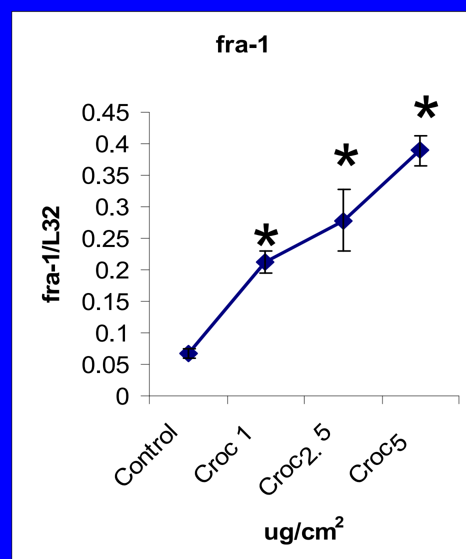
* $p<0.05$

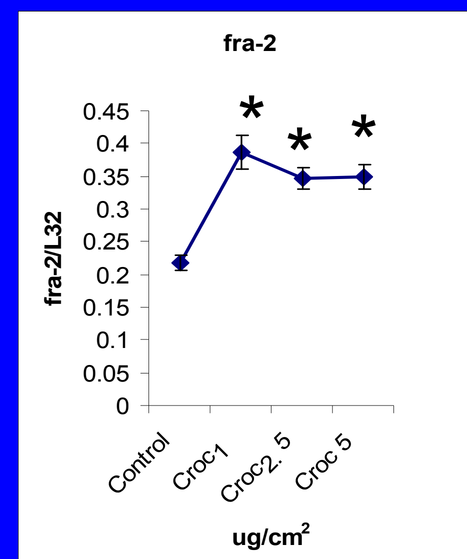












CAVEATS OF *IN VITRO* STUDIES

- Cell type-specific responses exist (Difficult to extrapolate doses/results from lab to lab).
- Do not reflect clearance/dissolution of fibers *in vivo* – SVF effects vs. Asbestos.
- Doses cannot be extrapolated to exposures or lung burden data in man.
- Doses (mass/area) often not useful for fiber type comparisons.
- Particle (non-pathogenic) controls often not included.

RAT INHALATION STUDIES

	Crocidolite		Chrysotile	
	High 8.25*[2800]	Low 0.16[60]	High 8.23[2457]	Low 0.18[32]
5'BrdU				
Bronch. Epith.	>(5d)	N.D.	>(5d)	–
Mesothelial	>(20d)	N.D.	>(5d)	–
c-jun	>	–	–	–
odc	>	–	–	–
Hydroxyproline	>	–	–	–

*Time-weighted average concentration (mg/m^3 air)
[ ] = Number of fibers > 5microns/cc air by PCM.

From Quinlan et al. Am J Path 147: 728-739, 1995; Quinlan et al., Am J Resp Crit Care Med 150: 200-206, 1994; BeruBe et al., Tox Appl Pharm 137: 67-74, 1996.

FIBER SIZE AND PROLIFERATION

- Long glass fibers (>5μ) are more potent than short fibers/particles in activating kinases (Ye *et al*., JBC 276:5360, 2001) or transcription factors.

- Compositional differences may also be important in long asbestos fiber stimulation of growth factor receptors (Pache *et al*., 1998).

FIBER SIZE AND PROLIFERATION

- Studies measuring proliferation in vitro by a number of markers (ODC, ^{3}H-thymidine, cell counts, metaplasia) show lack of or negligible effects of short fibers (<5μ) – Woodsworth *et al*., 1983; Marsh and Mossman, 1988.

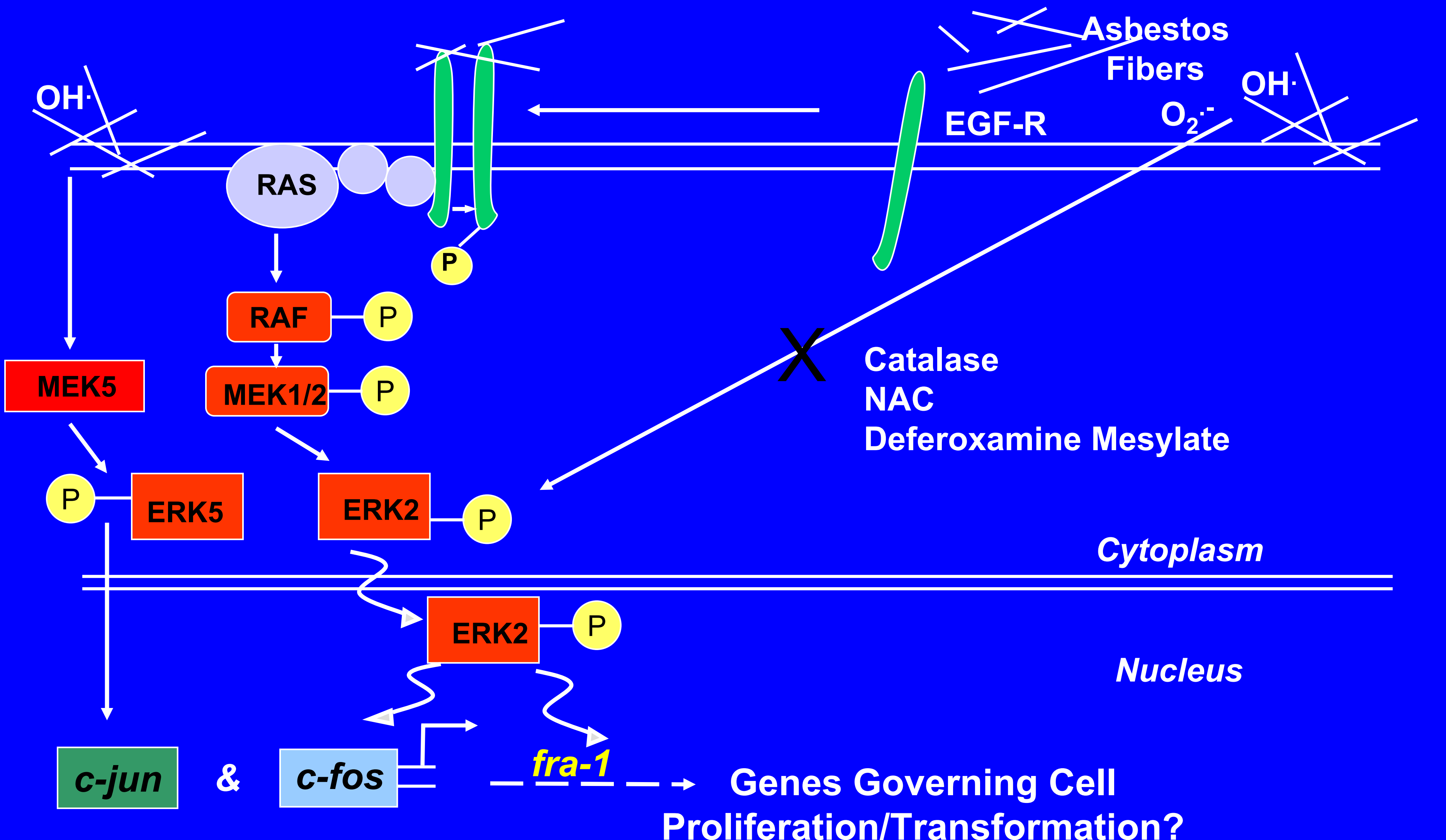
- Intratracheal instillation of long vs short crocidolite fibers in mice show lack of epithelial cell proliferation in response to short fibers (Adamson and Bowden, 1987 a, b).

CROCIDOLITE ASBESTOS AND CELL SIGNALING

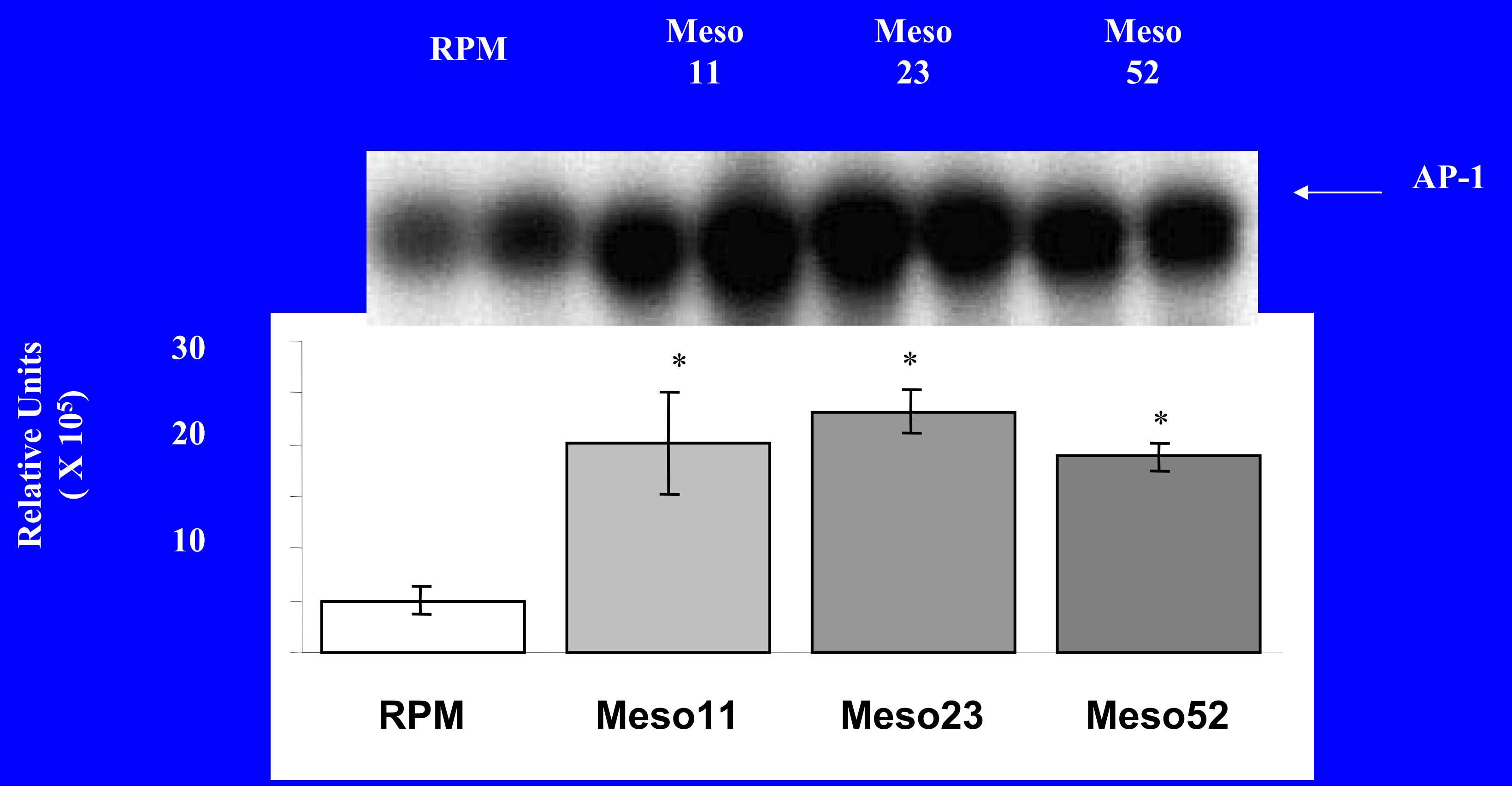
- Asbestos fibers interact with receptors (EGFR) to induce Extracellular Signal-Regulated Kinases (ERKs), phosphorylation and activity in pulmonary epithelial and mesothelial cells.

- Protracted increases in mRNA levels of various AP-1 member protooncogenes most importantly, ERK-dependent *c-fos*, *c-jun*, and *fra-1* are observed.

MECHANISMS OF ASBESTOS-CELL SIGNALING

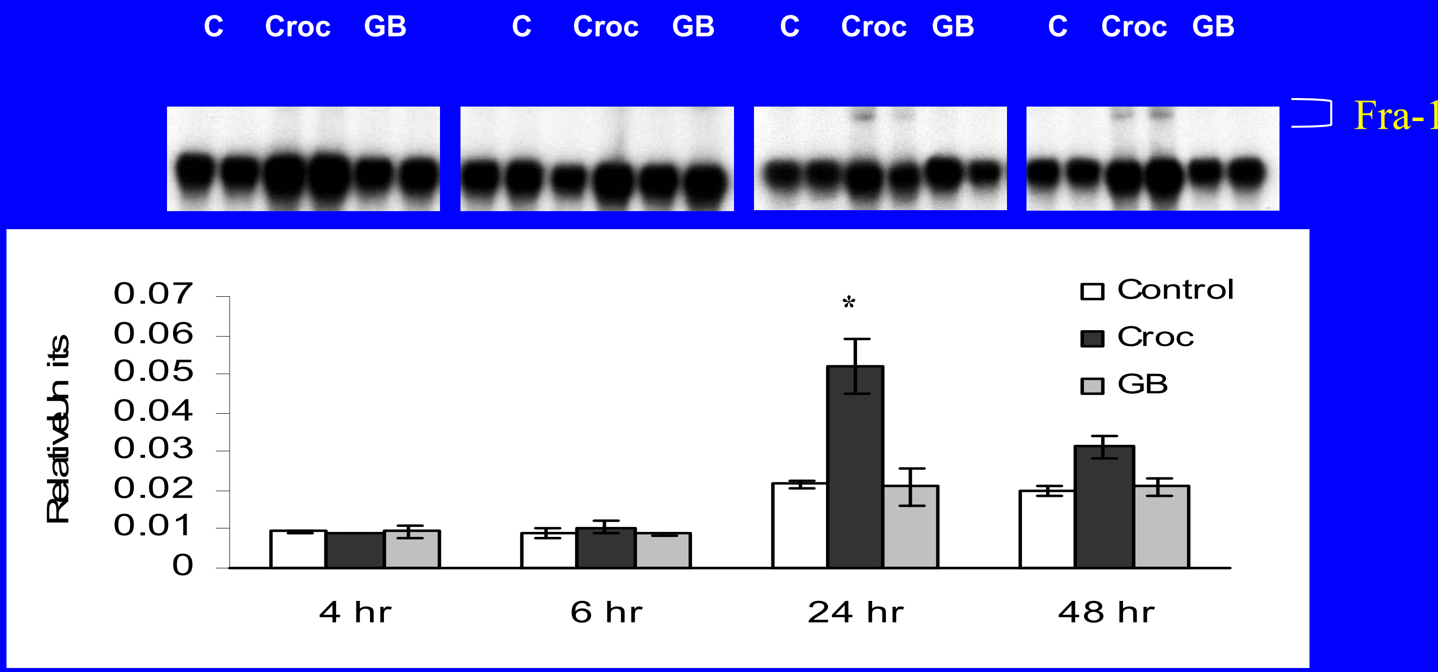


RAT MESOTHELIOMAS HAVE HIGHER LEVELS OF AP-1 THAN NORMAL RPM CELLS

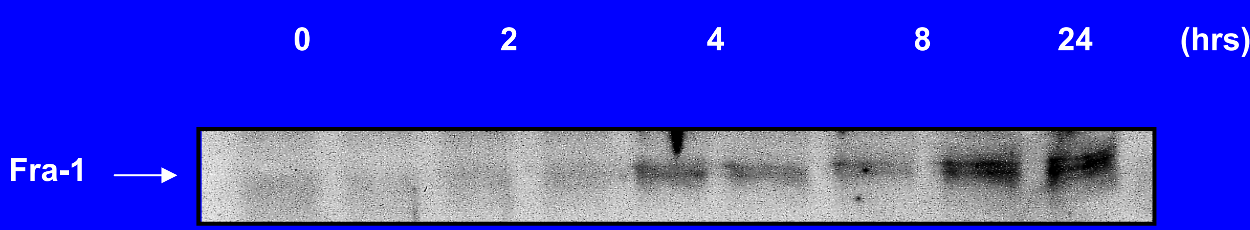


ASBESTOS EXPOSURE CAUSES PROTRACTED EXPRESSION OF FRA-1 IN RPM CELLS

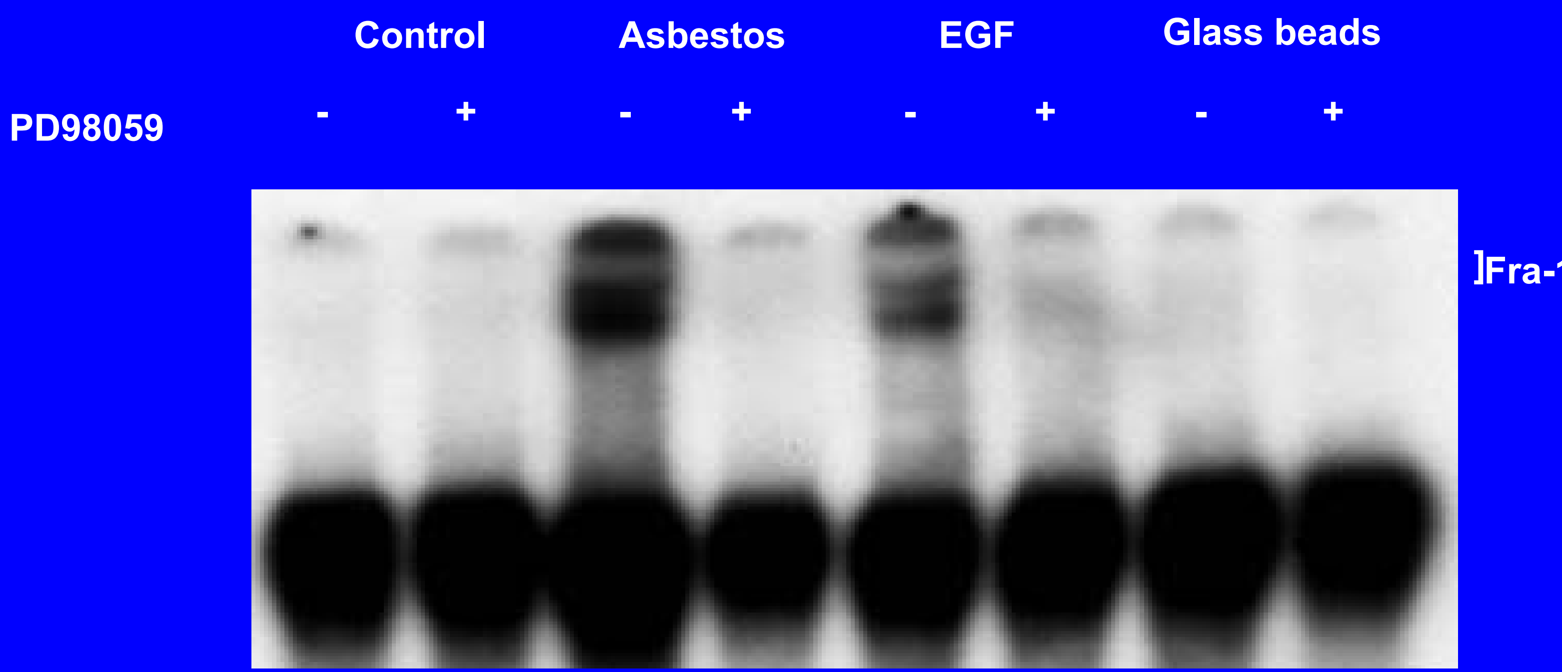
A.



B.



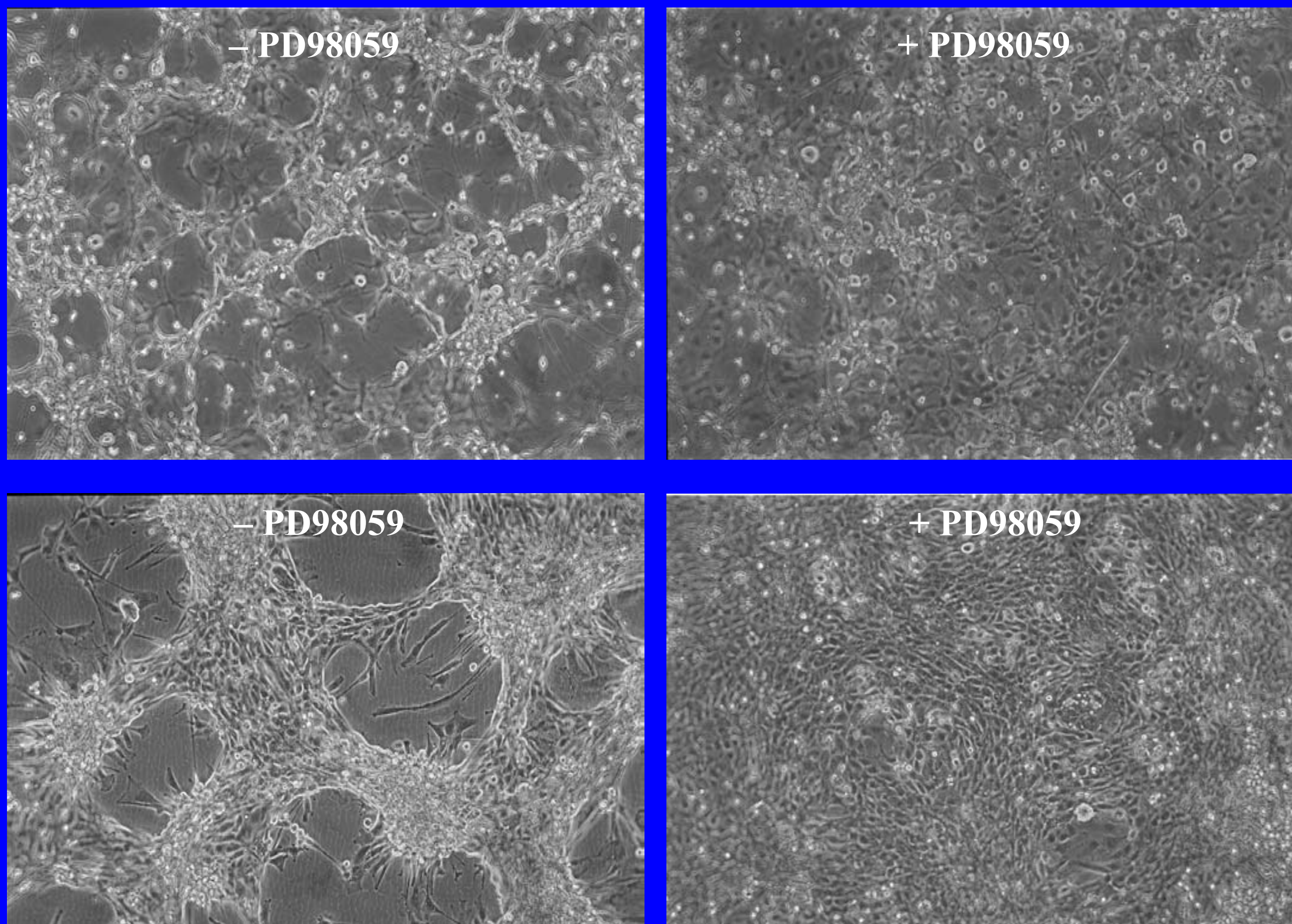
ERK-1 DEPENDENT FRA-1 IN AP-1 COMPLEXES IS INHIBITED BY THE MEK1 INHIBITOR, PD90859 (30μM)



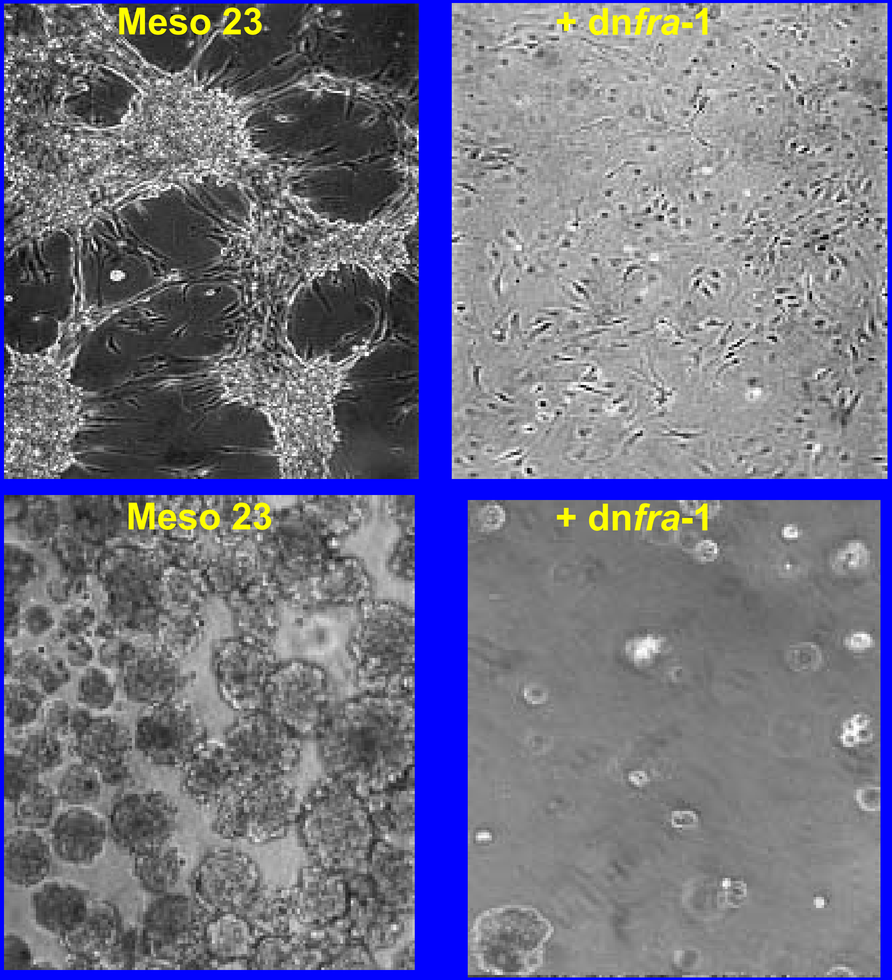
INHIBITION OF ERK-DEPENDENT FRA-1 REVERSES THE MALIGNANT PHENOTYPE OF RAT MESOTHELIOMAS

Meso 23

Meso 52



INHIBITION OF ERK-DEPENDENT FRA-1 REVERSES THE MALIGNANT PHENOTYPE OF RAT MESOTHELIOMAS



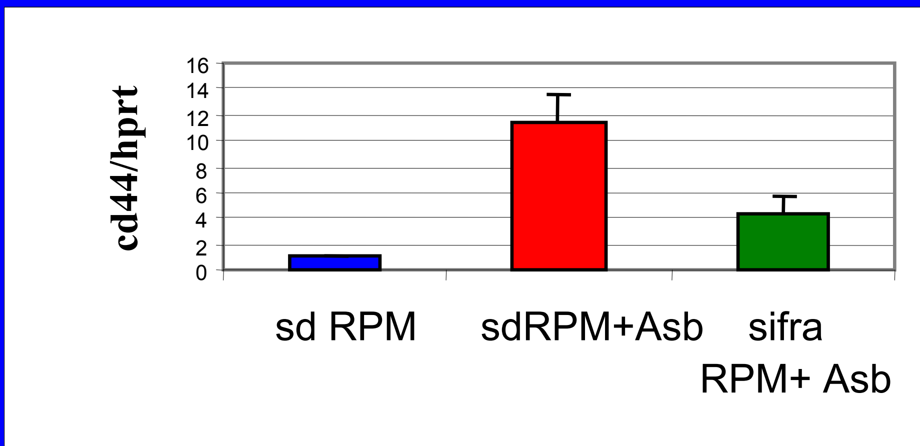
MICROARRAYS AND RNA INTERFERENCE (RNAi) LINK *fra-1* TO EXPRESSION OF MIGRATION/MOTILITY GENES

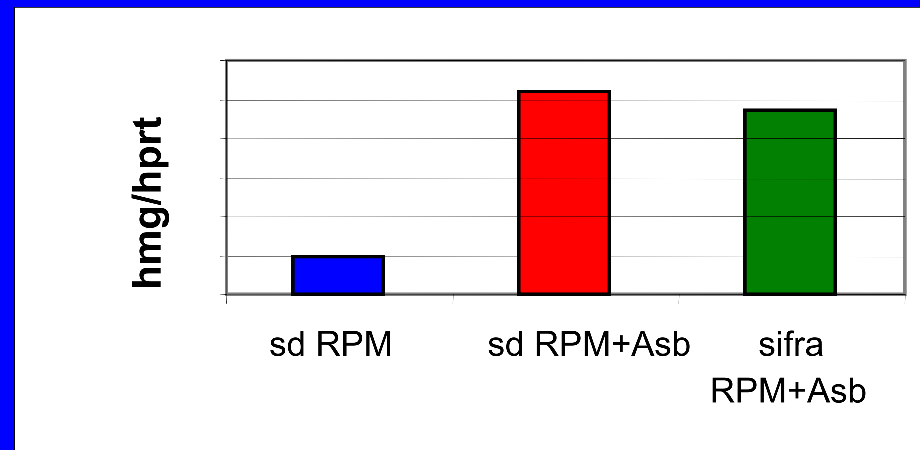
- Oligonucleotide microarray analysis (Affymetrix chip) on RPM, RPM exposed to crocidolite ($5\mu g/cm^2$) for 24 hr, 3 rat mesotheliomas.
- Selected gene expression, including *fra-1* confirmed by Real Time Q-PCR.
- RPM cells transfected (Oligofectamine) with an siRNA *fra-1* duplex or an siRNA scramble duplex (control) before Real Time Q-PCR on selected gene expression in response to asbestos.

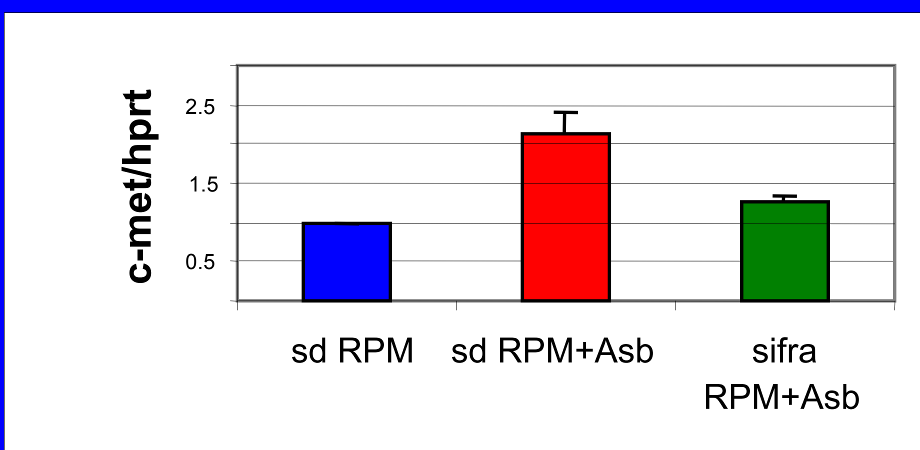
COMPARISONS OF CHANGES IN GENE EXPRESSION USING REAL TIME Q-PCR (TAQMAN) AND MICROARRAY ANALYSIS

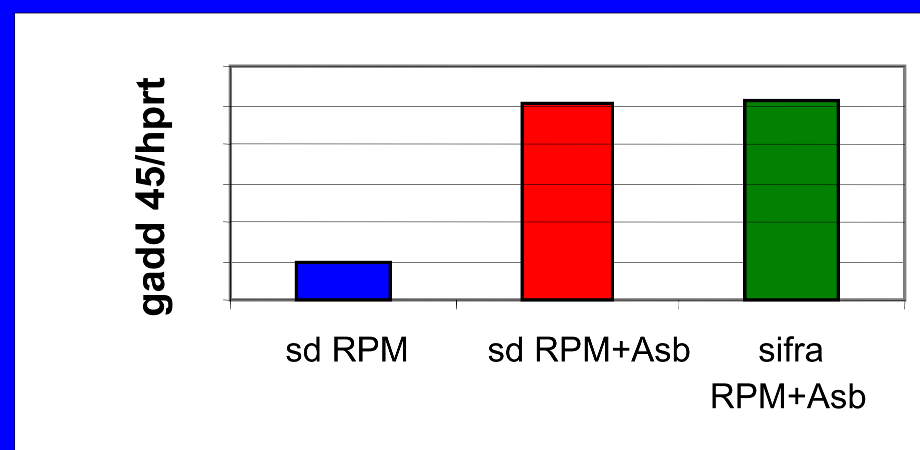
Gene	Method	RPM	Asb	Meso 11	Meso 23	Meso 52
fra-1	Taqman	1	9	219	117	311
	Microarray	1	10	289	319	677
cd44	Taqman	1	17	123	147	279
	Microarray	1	1.5	2	4	5
c-met	Taqman	1	3	7	16	9
	Microarray	1	2	30	54	19
High Mobility Group	Taqman	1	5	253	432	709
	Microarray	1	3	8	15	23
gadd45	Taqman	1	5	10	24	32
	Microarray	1	5	4	3	4

EFFECT OF siFRA-1 ON EXPRESSION OF SELECTED GENES UPREGULATED BY ASBESTOS IN RPM CELLS

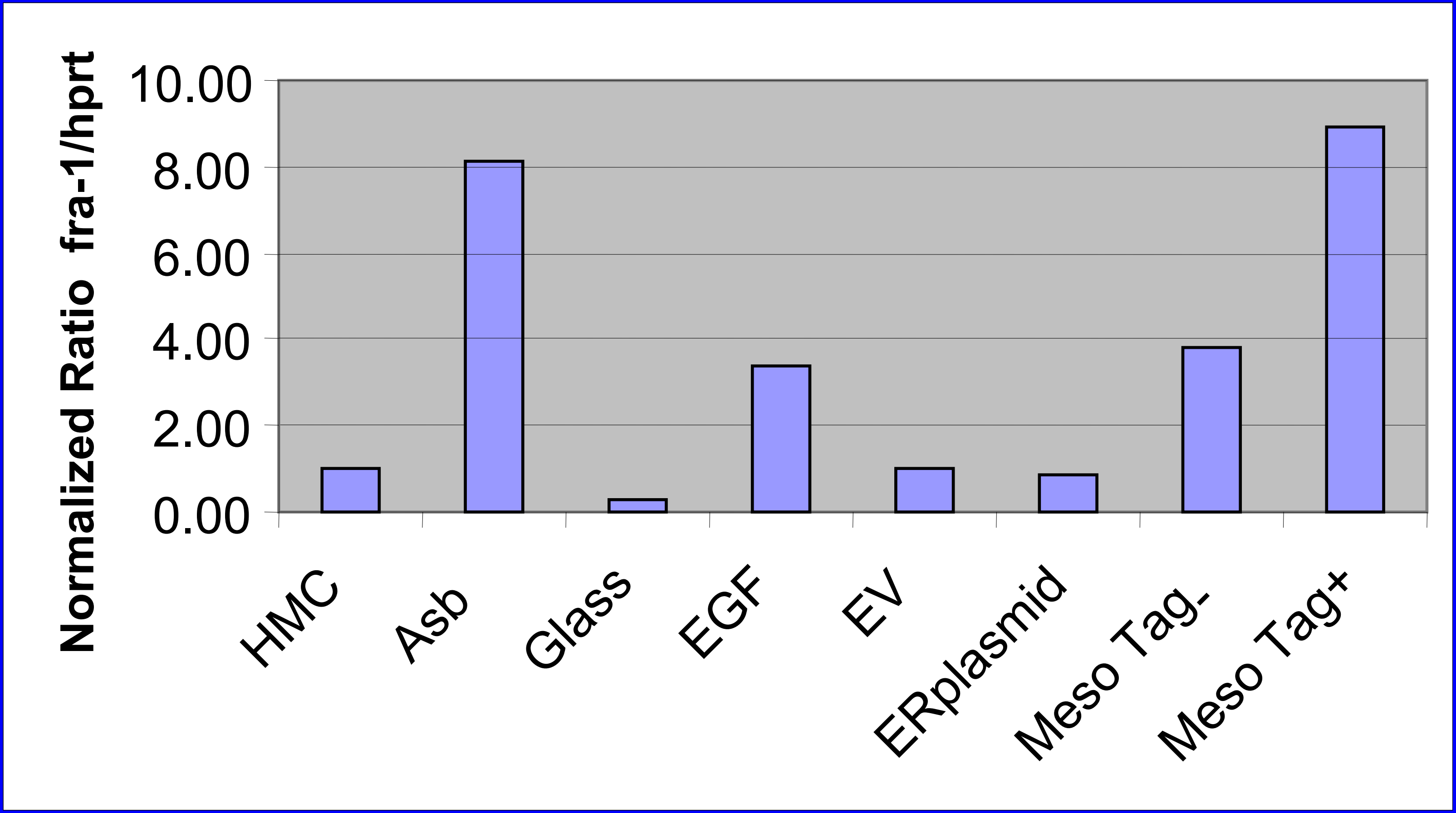




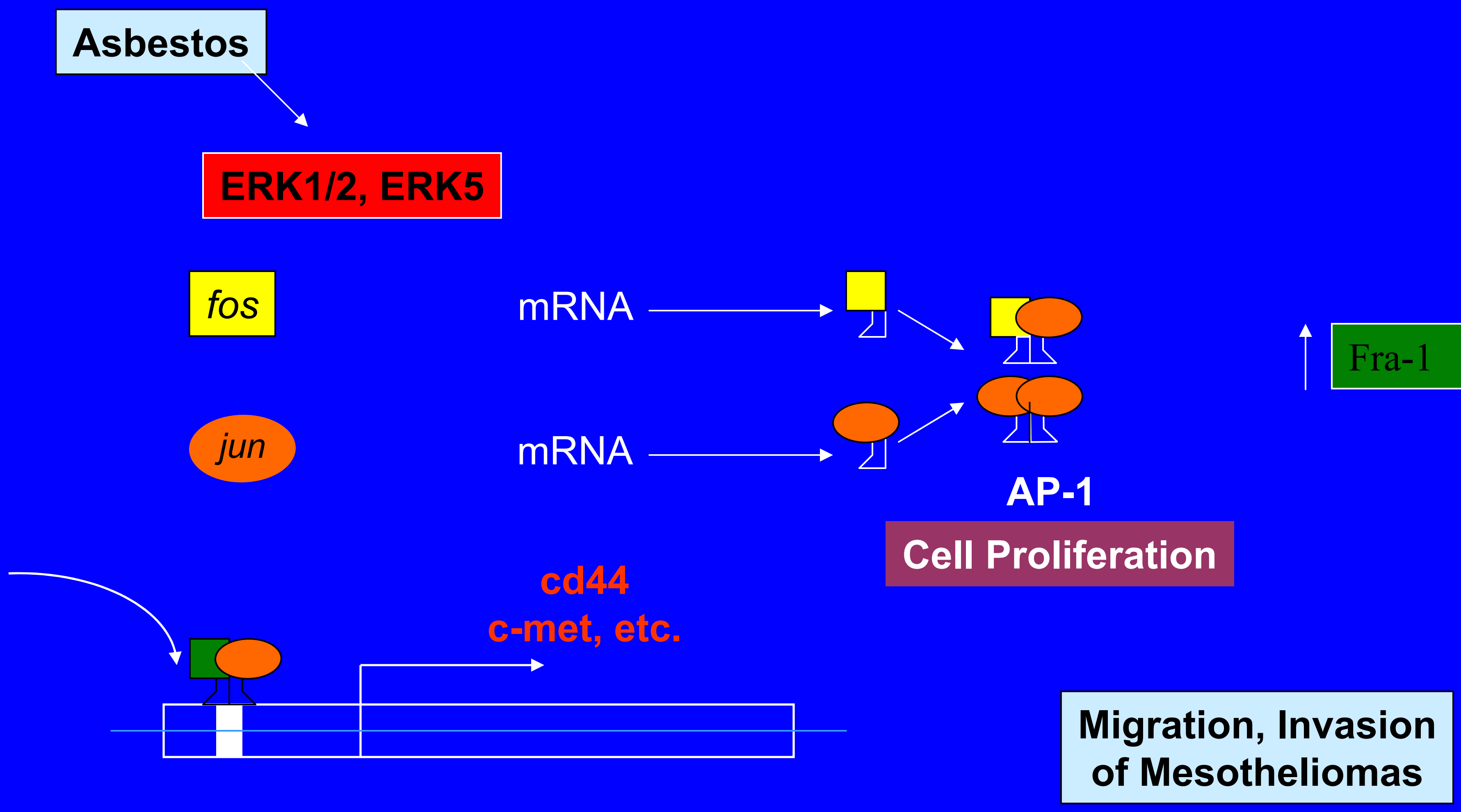




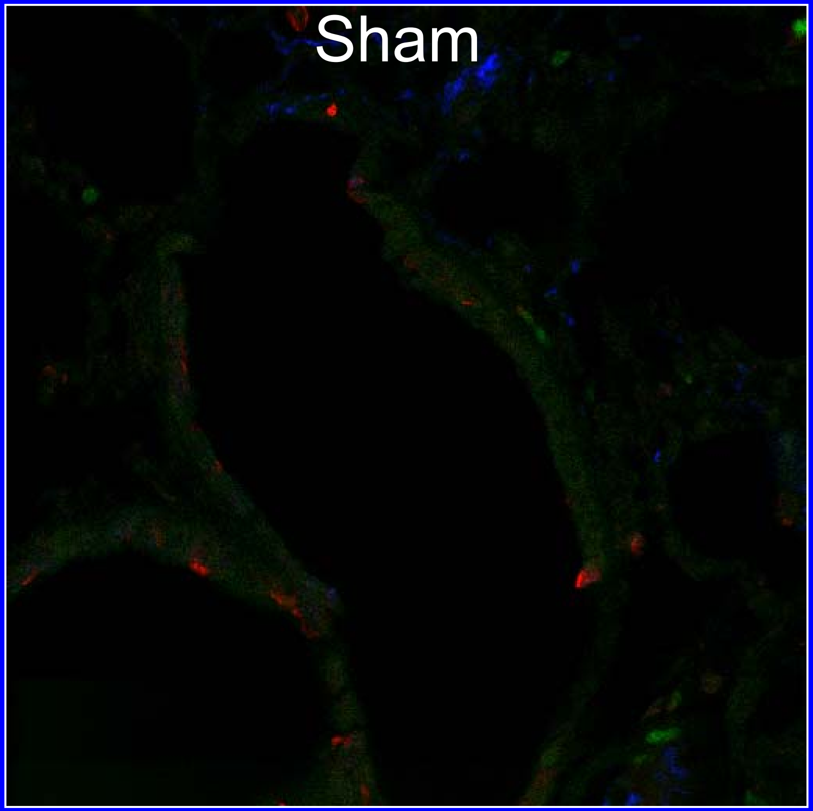
UPREGULATION OF *fra-1* IN HUMAN MESOTHELIAL CELLS (HMC) EXPOSED TO ASBESTOS AND IN MESOTHELIOMAS

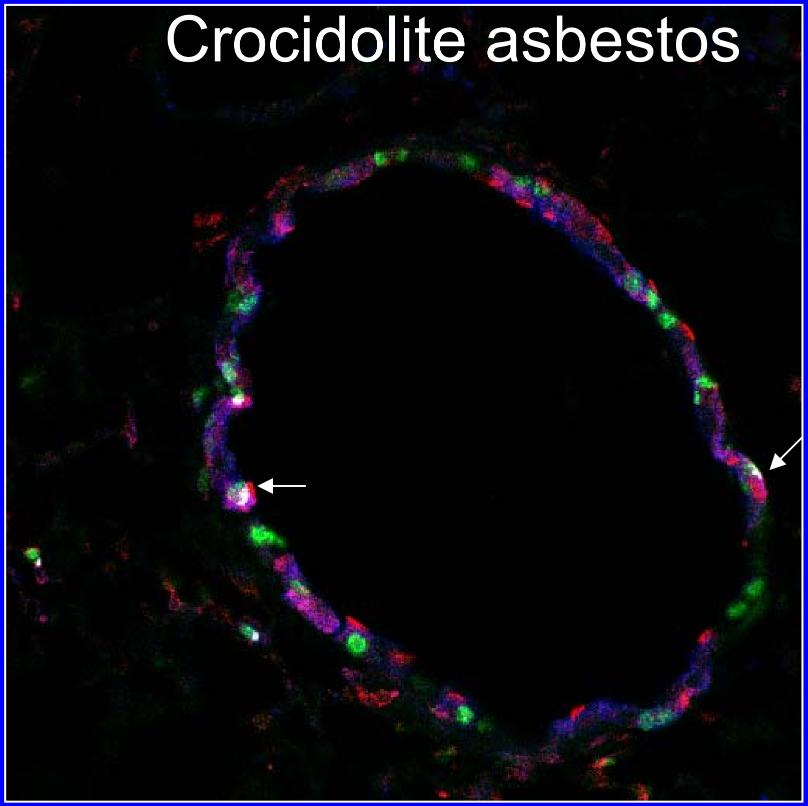


ASBESTOS SIGNALING, PROLIFERATION AND CARCINOGENESIS



TRIPLE LABELING OF SIGNALING PROTEINS IN MOUSE LUNGS





* mouse monoclonal PKCδ (blue), rabbit polyclonal p-ERK(red) and rat monoclonal Ki-67(green) antibodies

Mossman Laboratory

Maria Ramos-Nino, Ph.D.

Marcella Martinelli, Ph.D.

Luca Scapoli, Ph.D.

Cindy Timblin, Ph.D.

Arti Shukla, Ph.D.

Brian Manning, M.S.

Max MacPherson

Trisha Flanders

Astrid Haegans

Maria Stern

Collaborators:

Mauro Tognon, Ph.D.

Luciano Mutti, M.D., Ph.D.

Michele Carbone, M.D., Ph.D

Susan Land, Ph.D.

Nicholas Heintz, Ph.D.

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